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Chapter 4

Predicting the "first dose in children" of CYP3A-metabolized drugs: evaluation of scaling approaches and insights into the CYP3A7-CYP3A4 switch at young ages

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Abstract

First-dose-in-children relies on the prediction of clearance from adults for which little information is available on the accuracy of the scaling-approaches applied. For CYP3A-metabolized compounds, scaling of clearance is further challenged by different isoforms and by the CYP3A7 to CYP3A4 switch at young ages. This investigation aimed to evaluate the accuracy of two frequently used scaling-approaches and to gain insights into the ontogeny of CYP3A. Hence, a literature database was compiled containing 203 clearance values from term-neonates to adults for 18 CYP3A-metabolized compounds. The clearances in adults were scaled to children using i) allometric scaling *plus* maturation function and ii) a mechanistic approach based on the well-stirred model. Three maturation functions were separately evaluated. In children >3 months, all approaches were interchangeable heeding the maturation function applied and biases were mostly observed in children <3 months. The results from a sensitivity analysis indicate that these biases are possibly caused by disregarding the CYP3A7 activity which could account for up to 86% of the metabolism in term-neonates. Only the mechanistic approach using an overall-CYP3A maturation function led to unbiased predictions of clearances across all ages. The current investigation adds to the predictions of the first-dose-in-children of compounds (partially) metabolized by CYP3A.

Introduction

In the light of regulatory requirements to conduct pediatric clinical trials, emphasis has been placed on the selection of the first-dose-in-children which often relies on the scaling of clearance from adults to children. Two of the scaling approaches previously reported are (i) allometric scaling in combination with maturation of clearance for early life[1] and (ii) a mechanistic approach[2, 3]. The allometric scaling predicts total clearance in children by considering a 0.75-exponential relationship with body weight. In children younger than approximately 5 years, the gradual development of clearance is also attributed to developmental changes, which are described by a single maturation function. In contrast, the mechanistic approach is based on the concept of the well-stirred model for the prediction of clearance across various ages considering the ontogeny in key physiological processes, such as liver blood flow, plasma protein binding, liver size and maturation of the CYP3A enzyme activity in the gut and in the liver. This approach has previously been used as basis for physiologically-based-pharmacokinetic (PBPK) modules and combined with a population approach for prediction of clearance under disease conditions [4, 5].To date, the accuracy of both scaling approaches for prediction of the clearance in children has been evaluated to a limited extent for the mechanistic approach[2, 6], but never for the allometric scaling *plus* maturation function approach.

For compounds being metabolized by CYP3A enzymes, scaling of clearance is further hindered by the complexity of this metabolic pathway. The CYP3A subfamily is the most abundant group of cytochrome P450 isozymes in the liver and consists of at least three isoforms, i.e. CYP3A4, CYP3A5 and CYP3A7[7]. The different isoforms have different substrate-specificity and ontogeny[7,8,9] and are often simultaneously involved in the metabolism of pharmaceutical compounds[10]. In adults, compounds that are mainly metabolized by CYP3A4 are likely to be mainly metabolized by CYP3A7 in neonates and young infants[7, 8, 11]. Throughout development, the expression of CYP3A5 has been shown to be constant (the fraction of), polymorphically expressed and more limited in its metabolic capabilities than CYP3A4[11-13]. The exact and distinct characterization of the developmental expression of all these hepatic CYP3A isoforms is confounded by the lack of specific antibodies or specific markers for the enzyme activity and; by the often small sample size and the common practice of reporting results grouped over large age ranges[14, 15]. Altogether, it could explain the large varying and sometimes contradictory information available on CYP3A-ontogeny in the literature[2, 3, 16]. To the best of our knowledge, no information is available on how the widely varying public information on CYP3A-ontogeny impacts clearance predictions in children.

The current investigation aims to evaluate the accuracy of allometric scaling *plus* maturation function and the mechanistic approach for the prediction of the clearance in children using a large number of clearance values gathered from the literature for different CYP3A-metabolized compounds. In addition, three different CYP3A maturation functions previously reported to predict pediatric clearances[2, 3, 16] were evaluated. Further, a sensitivity analysis was performed to provide insights into the ontogeny of the CYP3A isoforms and into the relevance of the minor metabolic routes at young ages. Finally, this investigation allowed us challenging our previous findings that the maturation function used *plus* allometric scaling does not solely represent ontogeny of liver enzyme activity[17].

Methods

Literature database

From the literature, individual and mean clearance values in adults and children varying from term-neonates to adolescents were retrieved for a wide range of compounds reported to be primarily metabolized by CYP3A enzymes. Compounds known to undergo time-dependent changes in the pharmacokinetics and/or compounds of which pharmacokinetics is affected by the disease under investigation were excluded from the analysis. Additionally, information on age, body weight, blood-to-plasma concentration ratio and protein binding were collected. Mean age and body weight were used in case of mean clearances. For some compounds, information on the blood-to-plasma concentration ratio could not be identified in the literature and therefore the values were assumed to be 1. In case of oral administration, bioavailability and potential differences in relative bioavailability originating from the use of a pediatric formulation were also collected from the literature. For each compound an approximation of the extraction ratio was obtained using Equation 4.1.

$$Extraction\ ratio = \frac{CL_{adults} \cdot RB}{Q_{H_{adults}}} \qquad \text{Equation 4.1}$$

where CL_{adults} (mL/min) is the systemic plasma clearance in adults, RB is blood to plasma concentration ratio and $Q_{Hadults}$ (mL/min) is the hepatic blood flow in adults. The hepatic blood flow in adults and children was calculated from the sum of hepatic arterial and hepatic portal vein blood flow, representing 6.5% and 21% of cardiac output, respectively[3]. The cardiac output was calculated based on an anthropometric equation developed by Simone *et al* which has been previously reviewed by Price *et al*[18].

Scaling approaches

For every compound, the median clearance in adults was used to predict the clearance in children using two different scaling approaches: i) allometric scaling *plus* maturation function and ii) mechanistic approach, based on the well-stirred model of hepatic clearance. Demographics in adults and children were retrieved from the International Commission on the Radiological Protection[19].

Allometric scaling plus maturation function

The clearance in children was predicted using the following equation

$$CL_{children} = CL_{adults} \cdot \left(\frac{Weight_{children}}{Weight_{adults}} \right)^{0.75} \cdot f(Age) \qquad \text{Equation 4.2}$$

where CL_{adults} and $CL_{children}$ (mL/min) are the systemic plasma clearances in adults and children, respectively. Body weight in adults was assumed to be 70 kg. The *f(Age)* is the function of age representing the ontogeny of the CYP3A enzyme activity (maturation function).

Mechanistic approach

The mechanistic approach relies on the calculations of various derived physiological parameters prior to the prediction of clearance in children. First, intrinsic clearance in adults was calculated using the well stirred model (Equation 1.8) as represented in Equation 4.3.

$$CL_{int\ rinsic_{adults}} = \frac{CL_{adults} \cdot Q_{H_{adults}}}{fu_{adults} \cdot \left(Q_{H_{adults}} - CL_{adults} / RB\right)}$$ Equation 4.3

where $CL_{intrinsic,\ adults}$ (mL/min) is the intrinsic clearance in adults, CL_{adults} (mL/min) is the systemic clearance and fu_{adults} is the free fraction in plasma in adults. For orally administered compounds, the median of clearance was first corrected using the reported bioavailability.

Second, the intrinsic clearance per mg protein was calculated using the following equation:

$$CL_{int\ rinsic_{MMP}} = \cdot \frac{CL_{int\ rinsic_{adults}}}{LiverWeight_{adults} \cdot MPPGL}$$ Equation 4.4

where $CL_{intrinsic,\ MMP}$ (mL/min/mg) is the intrinsic clearance per mg protein and MPPGL is the microsomal protein per gram liver which was equal to 35 mg of microsomal protein per gram liver[3]. $LiverWeight_{adults}$ (g) is the liver weight in adults and was calculated using the anthropometric equation developed by Heinemann *et al* and reviewed by Price *et al* [18].

Subsequently, the intrinsic clearance in children ($CL_{intrinsic,children}$) was calculated using Equation 4.5.

$$CL_{int\ rinsic_{children}} = CL_{invivo} \cdot LiverWeight_{children} \cdot MPPGL \cdot f(Age)$$ Equation 4.5

where $CL_{intrinsic,children}$ (mL/min) is the intrinsic clearance in children, CL_{invivo} is the *in vivo* clearance and f(Age) is the function of age representing the maturation function that was used to characterize the ontogeny of the CYP3A enzyme activity. $LiverWeight_{children}$ (g) is the liver weight in children and was calculated using the anthropometric equation developed by Ogiu *et al* and reviewed by Price *et al*[18]. The concentrations of microsomal protein per gram liver (MPPGL) was assumed to be the same as in adults[3].

Finally, by rearranging Equation 4.3, the clearance in children was calculated using Equation 4.6.

$$CL_{children} = \frac{CL_{int\ rinsic\ children} \cdot Q_{H_{children}} \cdot fu_{children}}{Q_{H_{children}} + \left(fu_{children} \cdot CL_{int\ rinsic\ children} / RB\right)}$$ Equation 4.6

where $CL_{children}$ (mL/min) is the plasma systemic clearance in children, $Q_{Hchildren}$ (mL/min) is the hepatic blood flow in children and $fu_{children}$ is the free fraction in plasma. Developmental changes in free fraction in plasma were calculated by considering changes in the plasma protein concentration as described by Johnson *et al*[3]. In compounds with high extraction ratio, Equation 4.3 could not be applied as it led to negative values of intrinsic clearance in adults. For these compounds, Equation 4.6 was not used and the clearance in children was calculated by solely considering developmental changes in hepatic blood flow.

For orally administered compounds, plasma clearance predictions required prediction on the bioavailability in children which is influenced by age-related changes in the gut wall metabolism and first pass effect as described by Johnson *et al*[3].

Maturation of CYP3A activity

The allometric scaling and the mechanistic approach were separately investigated using in each instance three maturation functions of CYP3A activity in the liver. These maturation functions in combination with mechanistic and physiological approaches have been previously reported to adequately predict clearance in children[2, 3, 16].

The maturation of CYP3A reported by Edginton *et al*[2] comprises both the developmental changes of CYP3A4, CYP3A5 and CYP3A7 isozymes and was derived using *in vitro* and *in vivo* data. The maturation was reported as fraction of adult values per age and therefore interpolation was required to generate a continuous function enabling its use in the current investigation. Interpolation was performed using a generalized additive model. On the contrary, the maturation used by Johnson *et al*[3] comprises only the developmental changes of CYP3A4/5 and was derived using solely *in vitro* data. This maturation function is characterized by a *Hill* function as shown in Equation 4.7.

$$MF_{CYP3A4/5} = \frac{Age^{0.83}}{0.31 + Age^{0.83}} \qquad \text{Equation 4.7}$$

where $MF_{CYP3A4/5}$ is the maturation of CYP3A4/5 activity in the liver as a function of age.

Another maturation function evaluated in this investigation was the function used by Björkman *et al*. Björkman *et al* evaluated clearance predictions using a mechanistic approach using solely *in* vitro data from different substrates as reported by Lacroix *et al*[9]. In this investigation, only *in vitro* data representing the conversion from testosterone to testosterone 6β-hydroxylation was evaluated, as this data represents the activity of CYP3A4 with little activity of CYP3A5 and CYP3A7 in adults. As the exact maturation function used by Björkman *et al* in the predictions was not reported, the original data was retrieved from Lacroix *et al*[9] and used to estimate the percentage of adult values in relation to the median ages. A non-linear model was used to fit a sigmoid function (Equation 4.8).

$$MF_{CYP3A} = \frac{0.92 \cdot Age}{0.37 + Age} + 0.11 \qquad \text{Equation 4.8}$$

where MF_{CYP3A} is the maturation function of CYP3A activity in the liver.

Sensitivity analysis

A sensitivity analysis was performed to investigate the relevance of multiple CYP enzymes in the metabolism of mainly CYP3A-metabolized compounds in children. The maturation of multiple isoforms can be described as in Equation 4.9:

$$f(Age) = a \cdot MF_{CYP3A4} + b \cdot MF_{CYP3A7} + c \cdot MF_{others} \qquad \text{Equation 4.9}$$

where the sum of a, b and c equals 1 and represent the fractions metabolized by each isozyme in adults. For the selected compounds, in adults the metabolism occurs mainly via CYP3A4 and thus a>>b and a>>c. However, as CYP3A4 activity decreases with decreasing age, the relative contribution of other metabolic routes might increase. This was evaluated by plotting the ratio of the maturation of different CYP isozymes to the maturation of CYP3A4. The maturation functions of the multiple CYP isozymes (MF_{others}) have been previously reported by Johnson *et al*[3], except for the maturation

function of CYP3A7 (MF_{CYP3A7}). The latter maturation function has been derived from the *in vitro* data on dehydroepiandrosterone 16α hydroxylation reported by Lacroix *et al*[9]. A non-linear model was used to describe the ontogeny of CYP3A7 enzyme activity (Equation 4.10).

$$MF_{CYP3A7} = 14.0 - \frac{13.1 \cdot Age}{0.057 + Age} \qquad \text{Equation 4.10}$$

Further, simulations were performed to separately investigate the relative impact of CYP3A4 and CYP3A7 in the metabolism of CYP3A compounds across different ages. The maturation function from Johnson *et al*[3] was used as representation of the ontogeny of CYP3A4 activity (Equation 4.7) and the maturation function derived on the basis of the *in vitro* data reported by Lacroix *et al*[9] was used as a representation of the ontogeny of CYP3A7 activity (Equation 4.10). The maturation of the overall CYP3A activity used was either the one reported by Edginton *et al*[2] or derived using Lacroix *et al*[9] data. In these simulations, the overall maturation function of CYP3A was assumed to be the sum of the activity of CYP3A4 and CYP3A7, thereby disregarding the activity of potential minor metabolic routes (Equation 4.11).

$$f(Age) = a \cdot MF_{CYP3A4} + (1-a) \cdot MF_{CYP3A7} \qquad \text{Equation 4.11}$$

where a represents the fraction metabolized by CYP3A4 and (1-a), the fraction metabolized by CYP3A7 in adults. The value of a was estimated using the maturation function of CYP3A activity reported by Edginton *et al*[2] and Lacroix *et al*[9] as reference.

In all these simulations, the fraction of activity of CYP3A5 was assumed negligible and constant across all ages [11, 13].This makes the CYP3A4/5 maturation function previously reported by Johnson *et al*[3] representative to CYP3A4.

Graphical and statistical analysis

Graphical analyses were performed by assessing the ratio of the predicted clearances to the observed clearances *versus* age. Plots of predicted clearances following allometric scaling *plus* maturation function and using the mechanistic approach were compared for each of the maturation functions evaluated. Also, the percentage within two fold range and the average fold error were calculated for different age ranges using solely individual observations. The 2-fold range depicts the percentage of the observations within two fold of the median prediction and the average fold error depicts the log transformed ratio of the predictions to the observations (Equation 4.12).

$$afe = 10^{\frac{1}{N}\sum log\left(\frac{CL_{predicted}}{CL_{observed}}\right)} \qquad \text{Equation 4.12}$$

where afe is average fold error, N is the number of clearance values and $CL_{predicted}$ and $CL_{observed}$ is respectively the predicted and observed plasma clearance.

For all observed clearances in children, the median percentage error of the predicted clearance was calculated using Equation 4.13.

$$\%error = \frac{\left(CL_{predicted} - CL_{observed}\right)}{CL_{observed}} \cdot 100$$ Equation 4.13

Software

R version 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for the allometric scaling and mechanistic predictions as well as for graphical analysis. In addition, R packages gam (generalized additive models) and nls (non-linear least square) were used to fit the reported data on CYP3A activity in order to generate continuous maturation functions.

Results

Literature database

The compiled literature database comprised information of 18 compounds as shown in Table 4.1. These compounds were mainly low extraction ratio compounds. The calculated extraction ratio is an approximation of the "real" extraction ratio since it is strongly influenced specially by approximations of the blood-to-plasma concentration ratio which was not always available in the literature. The free fraction in plasma varies between compounds and slightly more than a half of these compounds were reported to mainly bind to albumin and the rest to alpha-1 acid-glycoprotein (AGP). These pharmacokinetic properties are potential factors for differentiation between the allometric scaling *plus* maturation function and the mechanistic approach.

Table 4.1 Pharmacokinetic properties of the compounds included in the database

Compound	Blood-to-plasma concentration ratio	Free fraction in adults	Protein	Calculated extraction ratio
Alfentanil	0.63	0.16	AGP	0.10
Amlodipine	1	0.025	Albumin	0.24
Etoposide	0.519	0.03	Albumin	0.02
Fentanyl	0.97	0.17	Albumin	0.70
Imatinib	0.83	0.05	Albumin	0.08
Indinavir	1.13	0.4	AGP	0.45
Itraconazole	1	0.01	Albumin	0.28
Midazolam	0.8	0.03	Albumin	0.31
Nifedipine	0.59	0.05	Albumin	0.09
Quinidine	1.84	0.16	AGP	0.27
Sildenafil	1	0.04	Albumin	0.40
Sufentanil	1	0.08	AGP	0.66
Tacrolimus	15.1	0.01	AGP	1.25
Tamoxifen	1	0.01	Albumin	0.02
Tamsulosin	0.53	0.02	AGP	0.02
Teniposide	1	0.01	Albumin	0.02
Triazolam	0.6	0.1	AGP	0.11
Zolpidem	0.76	0.08	Albumin	0.15

AGP = alpha-glycoprotein.

In adults, a total of 44 observed clearance values were collected from which 22 were mean values and 22 were individual values (Table S1). The median values for each compound in combination with the administration form were used to scale the clearance to children. In children from all ages, a total of 159 observed clearance values were available for comparison with calculated clearances (Table S2). More than half of these clearance values (64%) were individual reported clearances. In adults and children, data following intravenous and oral administration was well balanced. However, in children < 3 months most of the observed data was collected following intravenous administration in which systemic clearance predictions were not affected by potential misspecification in the scaling of the bioavailability. The data in this age group comprised in total 5 different compounds with low, intermediate and high extraction ratio including: alfentanil, etoposide, fentanyl, midazolam and sildenafil.

Maturation of CYP3A activity

Figure 4.1 shows the maturation functions of CYP3A activity applied in the current investigation. In children <10 days, the three maturation functions differed by approximately a factor two from each other. In children >10 years, the maturation function reported by Johnson *et al*[3] and derived from data reported by Lacroix *et al*[9] were similar. These two maturation functions markedly differed from the maturation function describing the data reported by Edginton *et al*[2] in children between 6 months and 5 years. In all cases, above the age of 5 years, the maturation was found to be at adult levels.

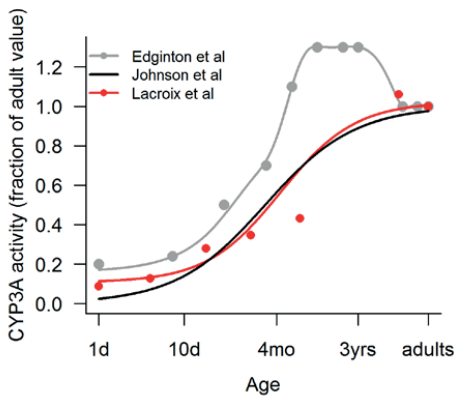

Figure 4.1 Maturation functions of CYP3A activity applied to the scaling approaches
The lines represent the maturation functions and the symbols the reported data.

Scaling approaches

Figure 4.2 illustrates the ratio of the predicted clearances to the observed clearances for different drugs *versus* age. In children >3 months, the predictive performance was visually similar for all scaling approaches applied. In addition, visual inspection showed that predictions in children >3 months were hardly influenced by the maturation functions used. In children <3 months, the predictive performance was strongly biased in all cases, except when using the mechanistic approach combined with the maturation function of CYP3A reported by Edginton *et al*[2] or the maturation function of CYP3A derived using data from Lacroix *et al*[9]. The maturation of CYP3A4 as reported by Johnson *et al*[3] showed the most biased results independent of the approach used.

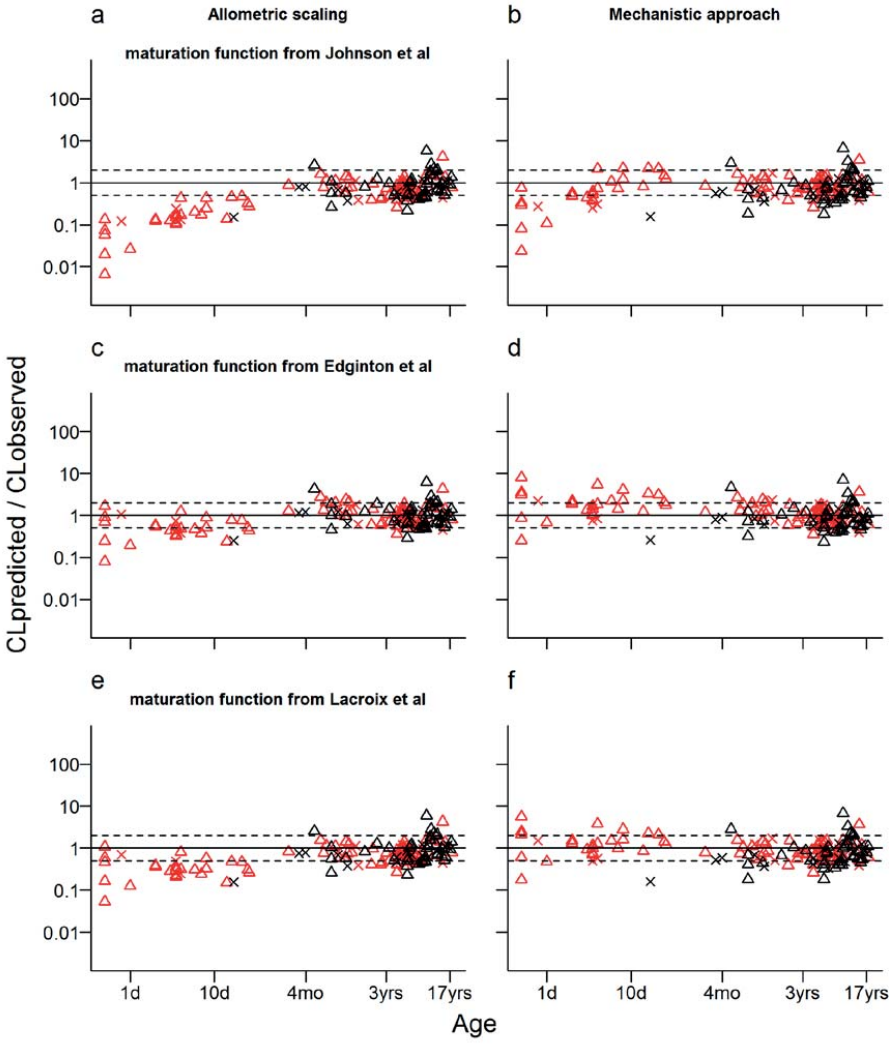

Figure 4.2 Predictive performance of the clearance scaling approaches when using different maturation functions
The left plots (a, c and e) represent the ratio of the predicted to the observed clearance when allometric scaling *plus* maturation function was applied. The right plots (b, d and f) represent the ratio of the predicted to the observed clearance when the mechanistic approach was applied. The upper plots (a and b) represent the results when using the maturation function reported by Johnson *et al*[3], the middle plots (c and d) represent the results when using maturation function reported by Edginton *et al*[2] and the lower plots (e and f) represent the results when using maturation function derived using *in vitro* data from Lacroix *et al*[9]. The symbols represent the observed clearance data after intravenous (red) and oral (black) administration. Individual data is represented by triangles and average data by x's. The dotted lines illustrate the two fold range.

These results are confirmed by the values of afe and the percentage 2-fold prediction error calculated in Table 4.2 for all ages and for different age groups. The age groups were selected following close examination of the results shown in Figure 4.1 and Figure 4.2:

- children >5 years as the maturation is in all cases at adult levels (Figure 4.1);

- children between 6 months and 5 years as the maturation from Edginton *et al*[2] differs from the other maturation functions by predicting CYP3A4 activity levels to be above adult levels in this age range (Figure 4.1);

- children >3 months and children <3 months as this age is a clear cut off during visual inspection of predictive performance (Figure 4.2).

Table 4.2 Predictive performance of the clearance scaling approaches when using different maturation functions for the CYP3A activity in the liver

Scaling approach	**Allometric scaling**	**Mechanism-based**	**Allometric scaling**	**Mechanism-based**	**Allometric scaling**	**Mechanism-based**
Maturation function	**Johnson *et al*[3]**		**Edginton *et al*[2]**		**Lacroix *et al*[9]**	
	0 – 18 years (N=125[a]/159[b])					
afe	0.54	0.75	0.87	1.1	0.67	0.9
2-fold range	54	68	73	74	60	73
	>5 years (N=58[a]/75[b])					
afe	0.85	0.82	0.96	0.91	0.88	0.85
2-fold range	64	71	78	74	67	69
	6 months – 5 years (N=38[a]/48[b])					
afe	0.72	0.75	1.1	1.1	0.74	0.77
2-fold range	79	82	84	87	82	84
	>3 months (N=97[a]/126[b])					
afe	0.81	0.81	1	0.99	0.83	0.83
2-fold range	69	74	79	78	72	74
	< 3 months (N=28[a]/33[b])					
afe	0.14	0.57	0.49	1.8	0.32	1.2
2-fold range	4	46	50	57	18	68

Only individual observed values were included in these calculations. [a] number of individual observed values; [b] total number of observed values

In children >5 years, all predictions were found comparable and unbiased. Slightly better results that were obtained when using the maturation function reported by Edginton *et al*[2] are likely to be due to the fact that of all three maturation functions investigated, this is the only one that predicts adult CYP3A4 activity to be equal to 1. Also in children between 6 months and 5 years, the most precise results were obtained using the maturation function reported by Edginton *et al*[2] (afe = 1.1 and 2-fold range = 87 %). In children >6 months, approaches seemed interchangeable when the same maturation function was applied. The most pronounced predictive performances were observed in children < 3 months. In this age group, scaling results were not interchangeable and allometric scaling *plus* maturation function always showed the worst results when compared to the mechanistic approach using the same maturation function. Unbiased results were only observed for the mechanistic approach in combination with the maturation function derived from Lacroix *et al*[9] data (afe = 1.2 and 2-fold range = 68%). This combination of scaling approach and maturation function was also shown accurate for the whole age range from 0 to 18 years (afe = 0.9 and 2-fold range = 73%) and across the age groups investigated.

In this investigation, individual observations had to be compared to average predictions for a certain age because literature information did not contain all the demographic information required for individual predictions. As a result, the higher percentage errors (>100%) observed is originated from individual observations (Table S2). Also, the great majority of the clearance values outside the 2-fold range predictions are represented by individual observations (Figure 4.2). For illustrative purposes, Figure 4.3 shows the prediction performance for fentanyl which although unbiased, contained observations outside the predicted 2-fold range. Also in Figure 4.3, an example of biased predicted performance is displayed using teniposide as an example.

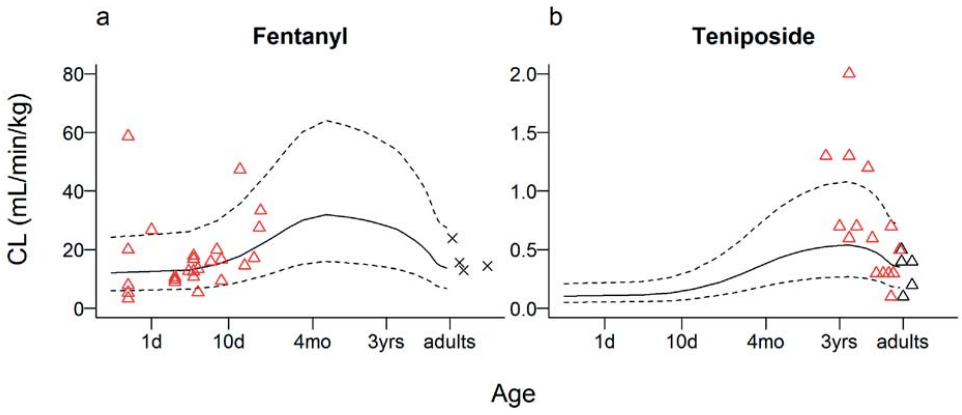

Figure 4.3 Predictive performance of the absolute clearance for fentanyl and teniposide when using the mechanism-based approach incorporating the maturation function derived using in vitro data from Lacroix *et al*[9].
The solid line represents the population prediction across different ages and the dotted lines the two fold range. Symbols represent the absolute clearances for adults (black) and children (red). Individual data is represented by triangles and average data by x's.

Sensitivity analysis for understanding the ontogeny of multiple CYP3A isoforms

Figure 4.4a illustrates the ratio of the maturation of the different CYP isozymes to the maturation of CYP3A4/5 activity. Most of the ratios decrease with increasing age and are shown to be the highest in neonates. The highest ratio observed is approximately 1300 and concerns the activity of CYP3A7 in relation to CYP3A4. The ratios of the maturation of the other CYP isozymes to CYP3A4 activity are lower than approximately 30. This indicates that even in neonates the activity of the other CYP isozymes is likely to be negligible when compared to the activity of CYP3A7.

Figure 4.4b illustrates the simulations performed to separately investigate the impact of CYP3A4 and CYP3A7 in the metabolism of CYP3A compounds across different ages. The activity of minor metabolic routes was disregarded in accordance with the results of Figure 4.4a. These simulations shows that in adults the relative activity of CYP3A7 is as low as 0.008 (1-a value in Equation 4.11) while in neonates this relative activity increases to up to 0.86 due to the increased activity of CYP3A7 in relation to CYP3A4. In addition, these simulations illustrate that the relative contribution of the activity of CYP3A7 is only relevant in children <3 months. The results were shown to be independent of the overall CYP3A maturation function used as reference.

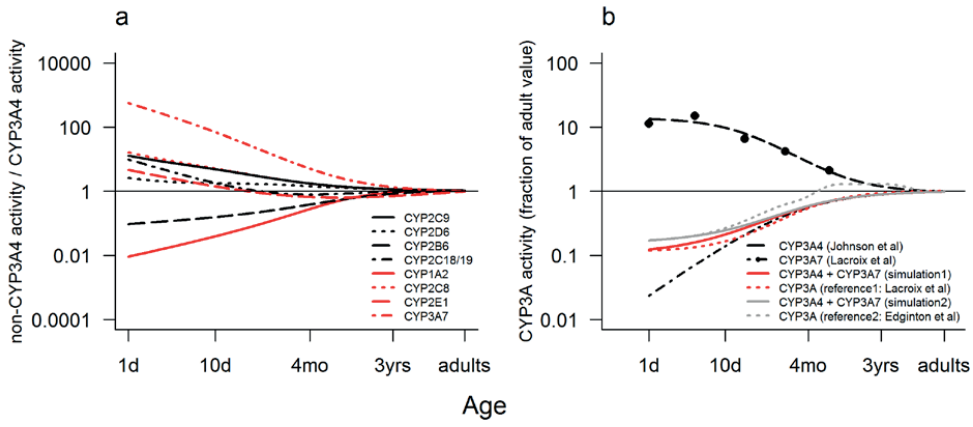

Figure 4.4 Sensitivity analysis to investigate the relevance of multiple CYP enzymes in the metabolism of mainly CYP3A metabolized compounds and the impact of CYP3A4/5 and CYP3A7 in the metabolism of CYP3A compounds across different ages
The left plot (a) illustrates the ratio of the maturation function of difference CYP enzymes to the maturation function of CYP3A4/5 as reported by Johnson *et al*[3]. The maturation function for CYP3A7 is derived from *in vitro* data published by Lacroix *et al*[9]. The right plot (b) illustrates the ontogeny of the activity of CYP3A7 using data from Lacroix *et al*[9] and CYP3A4 as described by Johnson *et al*[3]. The symbols are the in vitro data reported by Lacroix et al[9]. The simulated ontogeny activity of overall CYP3A was performed by adding up the activity of CYP3A7 and the activity of CYP3A4 as specified in Equation 18. The resulted simulated ontogeny was compared with the references described by Edginton *et al*[2] or derived using data from Lacroix *et al*[9].

Discussion

There is growing support by regulatory agencies to use scaling approaches for selection of the first-dose-in-children[20]. Frequently, the first-dose predictions rely on scaling of the clearance from adults for which various approaches can be applied. Two of the scaling approaches previously reported are allometric scaling *plus* maturation function[1] and the mechanistic approach[2, 3]. Very little information is known on the accuracy of these scaling approaches[2, 6]. Further, the prediction of clearance of CYP3A-metabolized compounds is confounded by the presence of different with distinct substrate-specificities and ontogenies that widely vary between references[2, 3, 16]. Altogether this leads to increased uncertainty in the clearance predictions. Hence, in this investigation we aimed to evaluate the accuracy and interchangeability of the allometric scaling *plus* maturation function and; of the mechanistic approach for CYP3A-metabolized compounds. For every approach, three CYP3A maturation functions previously reported to adequate predict pediatric clearances[2, 3, 16] were evaluated. Additionally, a sensitivity analysis provided insights into the ontogeny of the CYP3A isoforms and into the relevance of the minor metabolic routes at young ages.

For the retrospective evaluation, a literature database was compiled including 203 clearance values from term-neonates to adults of in total 18 compounds (Table S1 and Table S2) for which 79% of the total clearance values in children were individual clearances. The results of this retrospective evaluation showed that in children >5 years, the allometric scaling *plus* maturation function and the mechanistic approach provided accurate and interchangeable predictions, which most likely can be explained by the fact that maturation of enzyme activity in children >5 years is at adult levels. These results also show that the age-related developmental changes in key physiological processes influencing the clearance seems to be well captured by the allometric scaling function with an exponent of 0.75. Our results appear to be in line with conclusions from others that have shown that

in children >5 years clearance can be accurately predicted with a simple allometric function[6]. In children <5 years, predictions of clearance using allometric scaling requires it to be combined with a maturation function. Despite the widely application of this approach, its accuracy has never been determined.

This investigation illustrates for the first time the accuracy of allometric scaling *plus* maturation function. In children >6 months predictions were interchangeable between scaling approaches and within the same maturation function applied. However, in children <3 months, allometric scaling *plus* maturation function resulted in consistently lower clearance predictions (Figure 4.2) and accuracy (afe; Table 4.2) when compared to the mechanistic approach. A potential explanation for these results is that the maturation function used in combination with allometric scaling is not representative for ontogeny of the enzyme activity. This confirms the results of our previous investigation[17] where simulations have indicated that the maturation function aggregates multiple physiological and pharmacokinetic parameters which may include lipophilicity and extraction ratio. The mechanistic approach, on the other hand, considers extraction ratio, but disregards lipophilicity by assuming blood flow rather than permeation to determine the uptake in the liver. Nonetheless, the mechanistic approach combined with the maturation function derived from Lacroix *et al*[9] data resulted in unbiased clearance predictions across all ages. This accurate predictive performance was obtained without considering substrate-specificity, indicating that it can be neglected at least for the compounds evaluated.

The three CYP3A maturation functions evaluated were selected to be investigated as they have been reported to provide adequate predictions of clearance in children[2, 3, 16] notwithstanding differences in shape of the relationships (Figure 4.1). In children between 6 months and 5 years, the maturation function reported by Edginton *et al*[2] showed slightly better predictive performance for clearance (Figure 4.2; Table 4.2), showing that CYP3A activity around this age is indeed likely to exceed adult levels (Figure 4.1). In children <3 months, under-prediction of the clearance was observed when the maturation function reported by Johnson *et al*[3] was used (Figure 4.2; Table 4.2). Also in children <3 months of age, the maturation function reported by Edginton *et al*[2] and representing overall CYP3A activity led to over and under-prediction of clearance. Across all ages, accurate predictions of clearance were only observed when the maturation function derived from Lacroix *et al*[9] data was used in combination with a mechanistic approach (Table 4.2). In adults, this data essentially represents activity of CYP3A4/5 with little activity of CYP3A7, where in young children a CYP3A7 to CYP3A4 switch can be expected to influence the clearance. Further, similarities between this derived maturation function and the maturation function reported by Edginton *et al*[2] suggests that this data indeed represents overall CYP3A activity (Figure 4.1). This opposes initial postulation that it would mainly represent CYP3A4 activity[9] and supports the need for specific antibodies or specific markers for *in vitro* determination of the enzyme activity[14].

In order to gain more insights on the ontogeny of the CYP3A isoforms and more specifically on the switch of CYP3A7 to CYP3A4, a sensitivity analysis was performed. In this analysis the activity of CYP3A4 as described by Johnson *et al*[3] and the activity of CYP3A7 derived using data from Lacroix *et al*[9] were considered. The activity of overall CYP3A as described by Edginton *et al*[2] or as derived using data from Lacroix *et al*[9] were included as references to define the contribution fractions of CYP3A4 and CYP3A7 activity to the metabolism of different compounds (Equation 4.11). In line with this analysis, the extremely low activity of CYP3A7 in adults gradually increases with decreasing age to up

to 86% of the overall CYP3A activity in neonates (Figure 4.4b). Our results appear to be in line with independent *in vitro* results showing distinct patterns for the developmental expression for CYP3A4 and CYP3A7[14]. Additionally, the sensitivity analysis shows that the switch of CYP3A7 to CYP3A4 occurs within the first 3 months of life (Figure 4.4b). Interestingly, when the maturation function representing the activity of CYP3A4 as reported by Johnson *et al*[3] was used for the retrospective evaluation, biased clearance predictions were observed in children <3 months (Figure 4.2b). In addition, the relative activity of non-CYP3A4/5 metabolic routes in relation to the activity of CYP3A4/5 was explored. This showed that in children <3 months CYP3A7 activity overwhelmed potentially increased activity of minor non-CYP3A4/5 metabolic routes (Figure 4.4a).

There are two potential limitations in the current investigation. The first is related to the analysis results in children < 3 months which were based on a relatively small number of compounds (5 out of 18 compounds). However, these compounds displayed varying extraction ratios and this age group represented 21% of the total clearance values in children. The second is that the approaches evaluated were defined to predict mean clearance values in children, thereby ignoring existence of inter-individual variability. From a technical perspective, prediction of individual clearance is possible, but was hampered by the lack of (demographic) information at individual levels in both adults and children. As a result, predictions of the average clearance in children had to be compared with individual clearance values which although sometimes accurate (afe~1) for a certain age range still showed a great majority of the clearance values outside the 2-fold range predictions (Table 4.2 and Table S2). Obviously, in some cases the high 2-fold range predictions can be also the result of misspecification in model predictions or study differences in the collected data due to, for example, unreported differences in formulations or drug-drug interactions to co-medication. Both situations are respectively illustrated in Figure 4.3 which shows the predictive performance of fentanyl and teniposide. These results emphasize the need for future research evaluating the accuracy of the individual predictions.

In summary, the retrospective evaluation showed that the allometric scaling *plus* maturation function and the mechanistic approach were shown not to be interchangeable especially in children <3 months. Unbiased results across all ages were obtained using the mechanistic approach *plus* the maturation function derived from Lacroix *et al*[9]. In addition, the sensitivity analysis provided strong evidence that the activity of CYP3A7 should be considered when scaling the clearance to children <3 months, but also that an overall maturation function for the activity of CYP3A isoforms is sufficient to lead to accurate predictions. Further, the need of predicting inter-individual variability on clearances was highlighted. Altogether, this investigation adds to reduce uncertainty in the clearance predictions, thereby adding to the predictions of the first-dose-in-children of compounds (partially) metabolized by CYP3A.

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Supplement

Table S1 Observed data in adults used for scaling to children

Ref	Administration form (Intravenous/Oral)	Individual data? (Yes/No)	Age (years)	Weight (kg)	Observed bioavailability	Observed clearance (mL/min/kg)
	Alfentanil					
[1]	Intravenous	Yes	28	66	1	7.2
[1]	Intravenous	Yes	35	55	1	3.9
[1]	Intravenous	Yes	32	58	1	2.7
[1]	Intravenous	Yes	34	60	1	3.9
[1]	Intravenous	Yes	27	60	1	3.3
	Amlodipine					
[2]	Oral	No	30	70	0.77	5.9
	Etoposide					
[3]	Intravenous	No	30	70	1	0.643
	Fentanyl					
[2]	Intravenous	No	30	70	1	13
[4]	Intravenous	No	21.5	64	1	23.9
[5]	Intravenous	No	26.5	61.5	1	15.6
[6]	Intravenous	No	61	69	1	14.5
	Imatinib					
[7]	Oral	No	55	69	0.98	1.98
	Indinavir					
[8]	Oral	Yes	30	70	0.65	11.9
	Itraconazole					
[9]	Intravenous	No	30	70	1	5.4
	Midazolam					
[10]	Intravenous	No	22	69	1	7.58
[10]	Oral	No	22	69	0.24	40
	Nifedipine					
[11]	Oral	No	22	73	0.43	6.37
[12]	Oral	No	32.5	75	0.43	6.41
	Quinidine					
[13]	Oral	Yes	22	61.7	0.8	3.67
[14]	Oral	No	30	70	0.8	3.9
	Sildenafil					
[15]	Oral	No	57.7	86.6	0.41	11.3
[16]	Oral	No	30	70	0.41	23.7
[16]	Intravenous	No	30	70	1	9.71
	Sufentanil					
[17]	Intravenous	Yes	26	50	1	7.68
[17]	Intravenous	Yes	58	61	1	12.2
[17]	Intravenous	Yes	39	100	1	10
[17]	Intravenous	Yes	50	63	1	13.4
[17]	Intravenous	Yes	48	66	1	15.4

[17]	Intravenous	Yes	22	54	1	15.6
[17]	Intravenous	Yes	64	57	1	14.8
[17]	Intravenous	Yes	45	111	1	11.8
[17]	Intravenous	Yes	64	77	1	12.3
[17]	Intravenous	Yes	39	72	1	13.5
	Tacrolimus					
[18]	Intravenous	No	30	70	1	1.6
	Tamoxifen					
[19]	Oral	No	61	71	0.237	1.65
	Tamsulosin					
[20]	Oral	No	30	70	1	0.686
	Teniposide					
[21]	Intravenous	Yes	19	70	1	0.5
[21]	Intravenous	Yes	19	70	1	0.4
[21]	Intravenous	Yes	20	70	1	0.1
[21]	Intravenous	Yes	26	70	1	0.2
[21]	Intravenous	Yes	26	70	1	0.4
	Triazolam					
[22]	Oral	No	22.8	83.1	0.53	5.02
[22]	Oral	No	27	71.5	0.53	6.9
	Zolpidem					
[23]	Oral	No	27	71.5	0.7	5.3

Table S2 Observed data in children and percentage error in relation to the predicted clearance when using scaling approaches in combination with different maturation functions for the CYP3A activity in the liver

Ref	Administration form (Intravenous /Oral)	Individual data? (Yes/No)	Age (years)	Age (months)	Weight (kg)	Observed bioavailability	Observed clearance (mL/min/kg)	% error					
								Allometric scaling	Mechanistic	Allometric scaling	Mechanistic	Allometric scaling	Mechanistic
								Johnson *et al*[24]		Edginton *et al*[25]		Lacroix *et al*[26]	
	Alfentanil												
[27]	Intravenous	No	5	60.00	19.6	1	5.6	-30	-24	-6.8	-3.3	-28	-21
[28]	Intravenous	No	0.00962	0.12	3.66	1	1.7	-76	-52	-25	44	-51	-4.8
[29]	Intravenous	No	0.00962	0.12	3.66	1	3.2	-87	-75	-61	-24	-74	-50
[1]	Intravenous	Yes	4.7	56.40	20	1	3.4	14	21	54	56	18	25
[1]	Intravenous	Yes	5.5	66.00	22	1	5.9	-35	-32	-16	-15	-33	-30
[1]	Intravenous	Yes	7.7	92.40	23	1	8.3	-54	-48	-47	-42	-52	-46
[1]	Intravenous	Yes	4.5	54.00	14	1	4.6	-8.5	18	26	52	-5.2	22
[1]	Intravenous	Yes	4.8	57.60	24	1	3.7	-0.19	-2.4	35	25	3.5	0.57
[1]	Intravenous	Yes	4.5	54.00	20	1	4.8	-20	-15	10	9.8	-17	-13
[1]	Intravenous	Yes	6.2	74.40	23	1	4.4	-14	-8.2	8.1	10	-11	-5.5
[1]	Intravenous	Yes	4.9	58.80	22	1	2.7	40	44	88	83	45	48
	Amlodipine												
[30]	Oral	No	1.04	12.48	9.8	0.77	20	-63	-64	-37	-39	-62	-63
[30]	Oral	No	4	48.00	21.2	0.77	16	-55	-60	-37	-44	-53	-59
[30]	Oral	No	9.49	113.88	46.1	0.77	11	-41	-54	-37	-51	-39	-52
[30]	Oral	No	15	180.00	74.8	0.77	6.8	-17	-38	-14	-36	-14	-36
[30]	Oral	No	17.4	208.80	65.6	0.77	6.3	-7.9	-27	-5.3	-25	-5	-25
	Etoposide												
[31]	Intravenous	Yes	0.833	10.00	9.7	1	1.4	-43	-45	1.1	-4.6	-42	-45
[31]	Intravenous	Yes	0.958	11.50	8.5	1	1.4	-43	-39	-2.3	3.3	-42	-38
[31]	Intravenous	Yes	0.542	6.50	7.4	1	0.95	-22	-23	36	32	-23	-24

[31]	Intravenous	Yes	1.04	12.48	9.8	1	1.1	-25	-24	27	26	-24	-23
[31]	Intravenous	Yes	0.75	9.00	8.8	1	0.69	12	11	100	93	13	11
[31]	Intravenous	Yes	1.17	14.04	9.4	1	1.1	-22	-17	29	35	-21	-15
[31]	Intravenous	Yes	1	12.00	9.6	1	0.57	41	43	140	140	43	45
[31]	Intravenous	Yes	0.5	6.00	6.9	1	0.46	61	61	170	170	58	58
[31]	Intravenous	Yes	1.08	12.96	10.3	1	0.64	26	25	110	110	28	27
[31]	Intravenous	Yes	0.583	7.00	8.7	1	0.62	18	8	110	87	16	6.9
[31]	Intravenous	Yes	0.208	2.50	5.4	1	0.64	-11	-16	29	20	-17	-22
	Fentanyl												
[32]	Intravenous	Yes	0.00274	0.03	3.2	1	27	-97	-89	-81	-31	-87	-52
[32]	Intravenous	Yes	0.00137	0.02	3.3	1	20	-98	-92	-75	-12	-84	-39
[32]	Intravenous	Yes	0.00137	0.02	3.5	1	5.4	-93	-71	-9.2	210	-40	120
[32]	Intravenous	Yes	0.00137	0.02	4	1	59	-99	-98	-92	-74	-95	-82
[32]	Intravenous	Yes	0.00822	0.10	3.8	1	13	-87	-55	-56	35	-71	-6.4
[32]	Intravenous	Yes	0.0192	0.23	2.5	1	20	-83	-26	-62	44	-76	-0.3
[32]	Intravenous	Yes	0.011	0.13	2.1	1	5.5	-57	110	24	440	-19	280
[32]	Intravenous	Yes	0.0384	0.46	1.4	1	47	-86	-19	-76	25	-85	-13
[32]	Intravenous	Yes	0.00548	0.07	3.3	1	9.8	-87	-51	-44	90	-63	32
[32]	Intravenous	Yes	0.011	0.13	2.6	1	13	-83	-27	-52	85	-68	28
[32]	Intravenous	Yes	0.00137	0.02	2	1	3.4	-87	-24	66	700	9.6	460
[32]	Intravenous	Yes	0.00548	0.07	3.5	1	9.1	-87	-50	-41	95	-61	35
[32]	Intravenous	Yes	0.00548	0.07	2.5	1	11	-88	-43	-45	120	-64	53
[32]	Intravenous	Yes	0.00137	0.02	1.9	1	8	-94	-66	-29	250	-53	150
[33]	Intravenous	Yes	0.00962	0.12	3.66	1	11	-83	-39	-46	69	-65	17
[33]	Intravenous	Yes	0.00962	0.12	3.66	1	18	-90	-63	-68	1.3	-79	-30
[33]	Intravenous	Yes	0.00962	0.12	3.66	1	17	-89	-61	-66	8.5	-77	-25

[33]	Intravenous	Yes	0.00962	0.12	3.66	1	17	-89	-61	-66	8.5	-77	-25
[33]	Intravenous	Yes	0.00962	0.12	3.66	1	17	-89	-61	-66	7.9	-78	-25
[33]	Intravenous	Yes	0.00962	0.12	3.66	1	13	-85	-48	-54	44	-70	-0.53
[34]	Intravenous	Yes	0.058	0.70	1.88	1	17	-53	120	-24	210	-54	120
[34]	Intravenous	Yes	0.016	0.19	1.88	1	16	-80	5.6	-52	120	-69	54
[34]	Intravenous	Yes	0.022	0.26	1.88	1	9.4	-56	120	-9.7	310	-42	180
[34]	Intravenous	Yes	0.071	0.85	1.88	1	33	-73	25	-56	74	-74	21
[34]	Intravenous	Yes	0.068	0.82	1.88	1	28	-68	48	-49	110	-69	44
[34]	Intravenous	Yes	0.022	0.26	1.88	1	17	-75	25	-49	130	-67	59
[34]	Intravenous	Yes	0.044	0.53	1.88	1	15	-54	120	-22	230	-52	130
	Imatinib												
[7]	Oral	No	12	144.00	38	0.98	2.7	-19	-20	-16	-18	-17	-18
	Indinavir												
[35]	Oral	Yes	9.6	115.20	31	0.65	13	8.9	3.6	15	9.4	13	7.1
[35]	Oral	Yes	10	120.00	47.5	0.65	6.9	82	43	91	49	89	48
[35]	Oral	Yes	11.6	139.20	30	0.65	7.4	91	100	98	110	98	110
[35]	Oral	Yes	10	120.00	46.2	0.65	8.3	52	20	58	26	57	24
[35]	Oral	Yes	10.2	122.40	22.1	0.65	5.5	180	230	190	240	190	240
[35]	Oral	Yes	11.6	139.20	33.9	0.65	12	16	14	20	18	20	18
[35]	Oral	Yes	9	108.00	21.7	0.65	2.6	480	560	530	610	500	580
[35]	Oral	Yes	11	132.00	33	0.65	6.2	120	120	130	120	130	120
[35]	Oral	Yes	13.6	163.20	56	0.65	12	-2.8	-20	0.9	-17	0.43	-17
[35]	Oral	Yes	12.1	145.20	36.4	0.65	6.6	100	98	110	110	110	110
[35]	Oral	Yes	12.1	145.20	47.2	0.65	18	-29	-39	-26	-37	-26	-37
	Itraconazole												
[36]	Intravenous	No	1.4	16.80	9.1	1	19	-61	-50	-38	-26	-60	-49
[36]	Intravenous	No	4.2	50.40	18.2	1	8.8	-21	-13	10	13	-18	-10

[36]	Intravenous	No	9.5	114.00	28.8	1	10	-37	-33	-34	-30	-35	-31
[36]	Intravenous	No	14	168.00	55.1	1	13	-57	-62	-55	-61	-55	-61
	Midazolam												
[37]	Intravenous	No	5.2	62.40	18.4	1	12	-19	2.5	7	21	-16	4.8
[37]	Intravenous	No	4.7	56.40	15.9	1	8.5	17	57	59	89	21	61
[37]	Intravenous	No	1.3	15.60	8.8	1	9.1	11	68	80	130	13	71
[38]	Intravenous	No	0.8	9.60	7.8	1	11	-17	25	47	86	-16	25
[38]	Intravenous	No	5.3	63.60	19.5	1	10	-4.6	18	26	39	-1.1	21
[38]	Intravenous	No	15	180.00	60	1	9.3	-19	-27	-17	-26	-17	-26
[39]	Intravenous	No	0.00216	0.03	3	1	2.6	-88	-73	6.1	120	-30	51
[38]	Oral	No	0.8	9.60	7.8	0.24	50	-32	-31	21	-16	-31	-30
[38]	Oral	No	5.3	63.60	19.5	0.24	42	-17	-9	9.3	-18	-14	-5.7
[38]	Oral	No	15	180.00	60	0.24	25	8.9	22	12	-14	12	26
	Nifedipine												
[40]	Oral	Yes	0.42	5.04	2.38	0.43	3.6	170	190	330	370	160	180
[40]	Oral	Yes	0.67	8.04	6.37	0.43	33	-74	-82	-53	-67	-74	-82
[40]	Oral	Yes	0.67	8.04	6.86	0.43	8	5.4	-30	88	25	5.1	-30
[40]	Oral	Yes	0.67	8.04	6	0.43	15	-43	-59	1.8	-27	-43	-59
[40]	Oral	Yes	1	12.00	6.8	0.43	16	-43	-56	-2.7	-25	-42	-56
[40]	Oral	Yes	1.67	20.04	7.75	0.43	12	-19	-34	26	3.4	-17	-32
[40]	Oral	Yes	2.33	27.96	9.52	0.43	7.7	26	1.6	89	53	30	5
[40]	Oral	Yes	3.25	39.00	10	0.43	10	-1.8	-15	42	24	1.7	-12
[40]	Oral	Yes	5.67	68.04	20.7	0.43	8.6	-0.42	-28	29	-6.9	3.2	-25
	Quinidine												
[13]	Oral	Yes	3.7	44.40	14.4	0.8	9.5	-49	-50	-28	-30	-48	-49
[13]	Oral	Yes	8.7	104.40	25.7	0.8	9	-51	-54	-47	-50	-50	-53
[13]	Oral	Yes	8.8	105.60	30.9	0.8	9.3	-55	-61	-51	-58	-54	-60

[13]	Oral	Yes	8.8	105.60	26.7	0.8	7.4	-41	-46	-36	-41	-39	-44
[13]	Oral	Yes	9	108.00	24.2	0.8	4.6	-3.2	-4.5	4.5	3.1	0.16	-1.2
[13]	Oral	Yes	12	144.00	31.9	0.8	6.4	-35	-36	-33	-34	-33	-34
[13]	Oral	Yes	13.4	160.80	54.7	0.8	5.4	-32	-45	-30	-43	-30	-44
[13]	Oral	Yes	13.9	166.80	47.4	0.8	4.2	-8.8	-21	-5.5	-18	-5.8	-19
[13]	Oral	Yes	14.6	175.20	48	0.8	7.4	-49	-55	-47	-54	-47	-54
[13]	Oral	Yes	15.2	182.40	46.8	0.8	6.3	-39	-46	-37	-44	-37	-44
[13]	Oral	Yes	17.2	206.40	52.6	0.8	4.1	-8.7	-23	-6.1	-20	-5.8	-20
[13]	Oral	Yes	17.8	213.60	67	0.8	2.5	39	7.8	43	11	44	11
	Sildenafil												
[41]	Oral	No	0.332	3.98	4.8	0.41	25	-18	-38	22	-8	-22	-41
[41]	Oral	No	0.0466	0.56	2	0.41	61	-85	-85	-75	-74	-84	-84
[41]	Oral	No	0.277	3.32	5.1	0.41	24	-20	-44	15	-20	-24	-47
[42]	Intravenous	No	0.011	0.13	2.7	1	12	-87	-69	-62	-15	-75	-43
	Sufentanil												
[43]	Intravenous	No	2.08	24.96	12.1	1	18	-5.1	50	44	81	-2.1	52
[43]	Intravenous	Yes	2.58	30.96	16	1	41	-60	-44	-41	-34	-59	-43
[43]	Intravenous	Yes	2.67	32.04	11.3	1	40	-55	-25	-34	-11	-54	-24
[43]	Intravenous	Yes	3.25	39.00	16	1	34	-51	-31	-29	-19	-49	-30
[43]	Intravenous	Yes	3.83	45.96	14.2	1	30	-42	-11	-18	3.6	-40	-9.1
[43]	Intravenous	Yes	4.58	54.96	15	1	30	-42	-11	-20	1.1	-40	-9.9
[43]	Intravenous	Yes	4.58	54.96	19	1	20	-16	13	14	29	-13	15
[43]	Intravenous	Yes	4.83	57.96	17.5	1	45	-63	-47	-50	-40	-61	-46
[43]	Intravenous	Yes	5.25	63.00	18.5	1	45	-63	-48	-51	-42	-62	-48

[43]	Intravenous	Yes	5.42	65.04	17	1	18	-7	36	22	52	-3.6	38
[43]	Intravenous	Yes	5.75	69.00	14.5	1	19	-7.4	48	19	64	-4.1	51
[43]	Intravenous	Yes	5.92	71.04	28.5	1	24	-37	-28	-19	-20	-34	-26
[43]	Intravenous	Yes	5.92	71.04	25	1	26	-40	-27	-24	-19	-38	-26
[43]	Intravenous	Yes	6.92	83.04	29.6	1	33	-55	-48	-45	-44	-53	-47
[43]	Intravenous	Yes	7.5	90.00	23.5	1	28	-43	-26	-34	-21	-41	-25
[43]	Intravenous	Yes	7.5	90.00	15.2	1	40	-56	-28	-49	-23	-54	-27
[43]	Intravenous	Yes	8.75	105.00	22.6	1	35	-54	-38	-50	-35	-53	-37
[44]	Intravenous	No	11.7	140.40	44.7	1	16	-16	-13	-13	-12	-13	-12
	Tacrolimus												
[18]	Intravenous	No	8.2	98.40	25.9	1	2.1	-7.3	-7.5	3.9	3.7	-4.1	-4.2
	Tamoxifen												
[19]	Oral	No	6	72.00	27.2	0.237	3	-33	-67	-15	-59	-31	-66
	Tamsulosin												
[45]	Oral	No	8	96.00	25	1	0.7	19	22	35	38	24	26
	Teniposide												
[21]	Intravenous	Yes	4	48.00	17.4	1	2	-74	-74	-64	-64	-73	-73
[21]	Intravenous	Yes	2	24.00	13.4	1	1.3	-60	-62	-40	-42	-59	-60
[21]	Intravenous	Yes	4	48.00	17.4	1	1.3	-60	-61	-44	-45	-59	-59
[21]	Intravenous	Yes	7	84.00	24.2	1	1.2	-59	-60	-51	-52	-58	-58
[21]	Intravenous	Yes	5	60.00	19.6	1	0.7	-27	-28	-2.7	-4	-25	-25
[21]	Intravenous	Yes	4	48.00	17.4	1	0.6	-14	-15	21	19	-11	-12
[21]	Intravenous	Yes	4	48.00	17.4	1	0.6	-14	-15	21	19	-11	-12
[21]	Intravenous	Yes	3	36.00	15.6	1	0.7	-26	-27	8.2	5.7	-23	-25
[21]	Intravenous	Yes	8	96.00	26.9	1	0.6	-20	-22	-9.1	-12	-17	-19
[21]	Intravenous	Yes	9	108.00	29.4	1	0.3	58	52	70	64	63	57
[21]	Intravenous	Yes	11	132.00	37	1	0.3	50	39	55	43	55	43

[21]	Intravenous	Yes	13	156.00	47.6	1	0.3	42	24	47	29	46	28
[21]	Intravenous	Yes	15	180.00	55.8	1	0.3	37	16	41	19	41	19
[21]	Intravenous	Yes	15	180.00	55.8	1	0.3	37	16	41	19	41	19
[21]	Intravenous	Yes	14	168.00	52.6	1	0.1	320	260	330	270	330	270
[21]	Intravenous	Yes	14	168.00	52.6	1	0.1	320	260	330	270	330	270
[21]	Intravenous	Yes	14	168.00	52.6	1	0.7	-41	-49	-39	-47	-39	-47
[21]	Intravenous	Yes	18	216.00	70	1	0.5	-22	-40	-20	-38	-20	-38
	Triazolam												
[46]	Oral	Yes	8.17	98.04	23	0.53	18	-60	-64	-55	-60	-58	-63
[46]	Oral	Yes	6	72.00	20	0.53	6.9	10	-5.4	40	20	14	-2
[46]	Oral	Yes	5.83	69.96	29	0.53	6.9	0.45	-28	29	-8.3	4.1	-26
[46]	Oral	Yes	5.33	63.96	20	0.53	22	-66	-72	-55	-63	-65	-71
[46]	Oral	Yes	5.33	63.96	22	0.53	16	-55	-64	-40	-53	-53	-63
[46]	Oral	Yes	8.08	96.96	28	0.53	16	-56	-65	-51	-60	-55	-63
[46]	Oral	Yes	7.33	87.96	26	0.53	19	-63	-70	-56	-65	-62	-69
[46]	Oral	Yes	5.42	65.04	20	0.53	15	-50	-59	-35	-46	-49	-58
[46]	Oral	Yes	5.33	63.96	22	0.53	36	-80	-84	-73	-79	-79	-83
	Zolpidem												
[47]	Oral	No	4	48.00	17	4	12	-40	-43	-16	-20	-38	-41
[47]	Oral	No	8	96.00	30	8	9.7	-35	-42	-26	-35	-32	-40
[47]	Oral	No	16	192.00	58	16	4.8	15	-5.9	18	-3	18	-2.9

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